Research article

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The effects of three mash separation systems on the isomerisation of hop alpha-acids

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This study investigates the effects of wort composition from three lautering systems on hop utilisation at different hop boiling and dosing times. A response surface methodology was applied with 60 single tests at a 5 litre scale. The parameters, which were varied, were lautering system, boiling time without hops, boiling time with hops and α-acid dosage. It was shown that the wort composition from the different lautering systems requires different boiling times or enables the reduction in boiling time with hops. Although the pH and original gravity of the lauter tun and mash filter worts were similar, different boiling times were necessary to achieve the same concentration of iso-α-acids. Further, there were variations in fatty acid composition of the worts. In order to be able to assess the effects on a larger scale, six brews were performed in a 10 hL pilot brewery. The utilisation of hop bitter substances differed despite the same boiling time and the same α-acid dosage in relation to the total quantity of wort. In addition, no significant losses of hop bitter substances were observed in the wort from a continuous mash filtration system due to the process related higher dosage of α-acid. Both sets of experiments showed that the boiling times depend on the wort composition and increased as follows: novel continuous mash filtration system < mash filter < lauter tun. The results lay the foundation for calculating the optimal parameter settings for each brewery to optimise the hop isomerisation rate. © 2020 The Authors. Journal of the Institute of Brewing published by John Wiley & Sons Ltd on behalf of The Institute of Brewing & Distilling

Keywords: continuous mash filtration system; mash filter; lauter tun; hop utilisation; iso-alpha-acids; alpha-acids

Introduction

Beer production can take place in different ways, as the steps in the brewhouse may be performed with a variety of process equipment. One example is the lautering system, which separates the wort from the spent grains. Here, the two major systems used in breweries - the lauter tun and mash filter – were evaluated alongside a novel continuous system with rotating disc filter sieves *(1–4)*. Inevitably, these systems produce different worts, which are considered from the perspective of the rate of hop isomerisation.

During wort boiling, hop isomerisation is a key chemical reaction, where the hop acids are extracted into wort and isomerised to create the bitter iso-α-acids. However, there are losses in hop bitter substances during wort boiling. Parameters such as pH, original gravity, wort composition, α-acid dosage and hop boiling time influence the rate of isomerisation *(5,6)*. Previous results showed that hop boiling times have to be adjusted accordingly for worts of different original gravities and pH *(7)*. Therefore, it is necessary to investigate how different worts, produced by different lautering systems, influence the hop isomerisation rate during wort boiling. Such results can lay the foundation for the development of a tool for the calculation of the amount of hops to be added and the associated boiling times for different wort qualities.

It is important to note, that the lautering systems cannot be considered alone. For example, a lauter tun needs a different grist composition to a mash filter, which can process a finer grist. Further, the continuous mash filtration system simultaneously separates the wort with four pairs of rotating disc filter sieves. In contrast to the static filtration in lauter tuns and mash filters, dynamic filtration without the formation of a filter bed is used in the continuous system. Additionally, the modules of the system are in cascade arrangement, offering the possibility to sparge in the transition between the separate modules in order to further extract the malt and gain more extract. Four wort flows with different characteristics, e.g. pH, wort composition, are produced in parallel due to the three sparging steps and the four-step separation process. This leads to a different brewhouse design in order to optimally use the four parallel wort flows (Figure 1, bottom row). For example, a first wort extract from the first module ensures post-saccharification after wort boiling at 83°C, and the weak wort of the fourth module (<2.0°P) improves hop isomerisation in a separate vessel *(7–10)*.

The experiments at a 5 litre laboratory scale were developed using response surface methodology (RSM) to evaluate the isomerisation rate in worts using different lautering systems by varying α-acid dosage, hop dosing time and different boiling time. In order to be able to extend these results to a commercial scale, these tests were extended to a pilot brewery scale.

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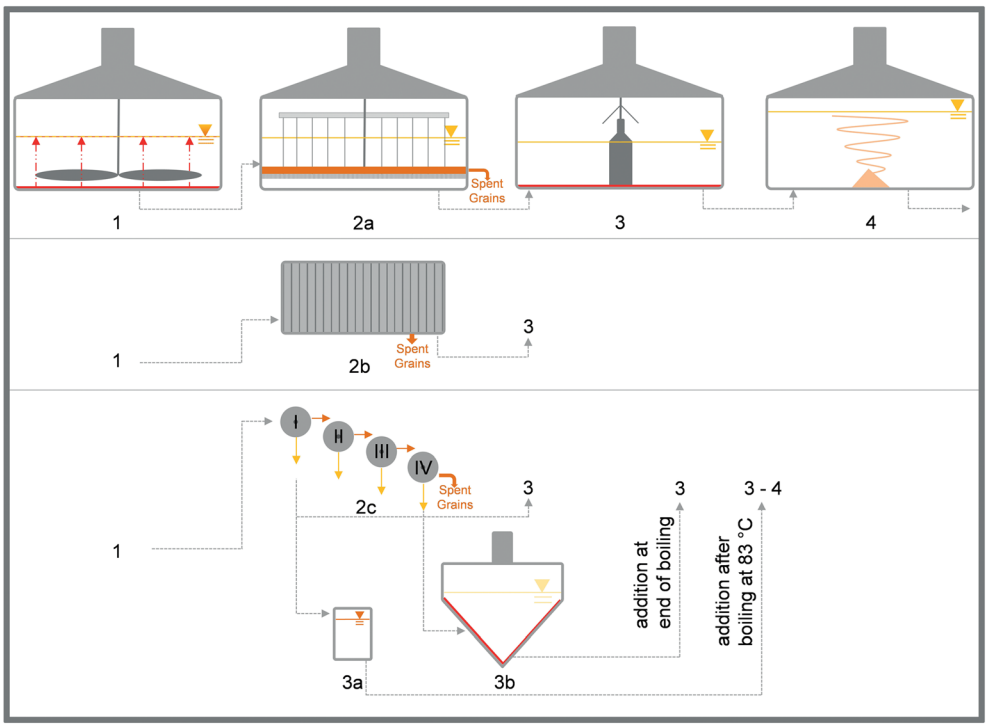

Figure 1. Wort production scheme with different liquid-solid separation systems. 1. mashing vessel, 2a. lauter tun, 2b. mash filter, 2c. continuous mash filtration system, 3. wort kettle, 3a. malt extract, 3b. separate isomerisation vessel, 4. whirlpool. [Colour figure can be viewed at wileyonlinelibrary.com]

Materials and methods

Design of experiments

The plan for the laboratory scale experiments (5 L) was generated with Design Expert® (version 10, Stat-Ease, Inc., MN, USA). For this, a response surface – central composite rotatable design was applied. Four factors were included (Table 1): boiling time without hops (BTW in minutes), hop boiling time (HBT in minutes) and α-acid dosage (mg/L) as numeric factors as well as lautering system as categoric factor. Each numeric factor had 5 settings. For the categoric factor, there were three settings: mash filter, lauter tun and continuous mash filtration system. In total, 60 trials were performed. As response data, the bitterness units (BU), α-acids (mg/L), β-acids (mg/L) and iso-α-acids (mg/L) were analysed in the boiled wort.

A total of 6 brews were performed at pilot brewery scale (10 hL), 2 brews for each lautering system. The utilisation of bitter substances (Eq 1) was calculated at the end of hop boiling.Eq 1

$$utilisation\ \% = \frac{IAA\ in\ cast\ out\ wort}{AA\ added} \cdot 100 \qquad \text{(Eq1)}$$

where:

IAA	:	iso-α-acids (mg/L)
AA	:	α-acids (mg/L)

Statistical evaluation

The response data were utilised to calculate the statistical evaluation of the series of experiments. The statistical significance of the factors and interactions were determined using Analysis of Variance (ANOVA) and the software Design Expert® (version 10, Stat-Ease, Inc., MN, USA).

To evaluate the response data of the laboratory scale experiments, a reduced cubic model was chosen according to the p-values of the parameters and the all hierarchical models

Table 1. Factors selected for experimental design generation with their specific settings and coded levels in parentheses.

numeric factor			categoric factor
boiling time without hops (min)	hop boiling time (min)	α-acid dosage (mg/L)	system
0 (-α)	40 (-α)	60 (-α)	lauter tun
8 (-1)	62 (-1)	88 (-1)	mash filter
20 (0)	95 (0)	130 (0)	continuous mash filtration system
32 (+1)	128 (+1)	172 (+1)	-
40 (+α)	150 (+α)	200 (+α)	-

Table 2. Overview of the applied response transformation as well as the model data for the responses from a central composite rotatable design experiment.

responses	iso-α-acids	α-acids	β-acids	bittering units
response transformation	none	square root	square root	none
R^2	0.99	0.94	0.98	0.99
adjusted (adj.) R^2	0.98	0.88	0.96	0.98
predicted (pred.) R^2	0.93	0.73	0.87	0.93
model p-value	<0.0001	<0.0001	<0.0001	<0.0001
model F-value	92.70	16.30	53.75	103.82
lack of fit F-value	1.97	0.99	1.49	2.09

procedure. This removed the combination options of higher order. Table 2 shows the information for the response transformation. As the variance was proportional to the mean, the square root transformation for the results of α- and β- acids was applied. This transformation achieves a normal distribution and enables the analysis of variance *(11)*. The evaluation of the data was executed with a two-sided confidence interval of 0.05. Table 2 illustrates the calculated coefficients of determination as well as the adapted and the predicted coefficients of determination. Adjusted R^2 should be close to R^2, since it adapts R^2 to the number of parameters in the model and the number of points in the design. How well a regression model can provide predictions is indicated by the predicted R^2 *(12)*. If a model p-value is equal to or less than the significance level, then the model is applicable. The F-value considers the ratio of the variance of the model to the variance of the residuals. Consequently, with similar variances, the errors in the model are as large as the actual effect of the model. Thus, the model would not be applicable *(12)*.

Laboratory scale experiments

In order to perform the laboratory scale experiments (5 litres), the worts were produced according to the scheme below. The required volume of unboiled, unhopped wort was separated from the process at 95°C. The samples were directly processed in the laboratory and not stored. A total of 15 brews, 5 per lautering system, were performed and sampled. In the lauter tun and mash filter trials, the worts were collected from the wort kettle (Figure 1, '3'). In the trials with the continuous mash filtration system, the wort was taken from a separate isomerisation vessel (Figure 1, '3b'). Subsequently, the parameters, boiling time without hops, boiling time with hops as well as the α-acid dosage, were adjusted according to the experimental design. Each of the settings was repeated for the three worts. After the corresponding boiling time, the worts were cooled and transported (5°C) to the Research Center Weihenstephan for Brewing and Food Quality for analysis.

Wort production

Wort production for the trials (laboratory and pilot plant scale) was in the fully automated 10-hL pilot brewery of Ziemann Holvrieka GmbH (Ludwigsburg, Germany). The Pilsner barley malt from Mich. Weyermann GmbH (Bamberg (Germany), was ground with a 6-roller mill for the trials with the continuous mash filtration system and lauter tun. The grist for the membrane mash filter trials was ground with a hammer mill, in accordance with the requirements of the system. 175 kg malt were used for mashing-in with the lauter tun and continuous mash filtration system and 85 kg malt

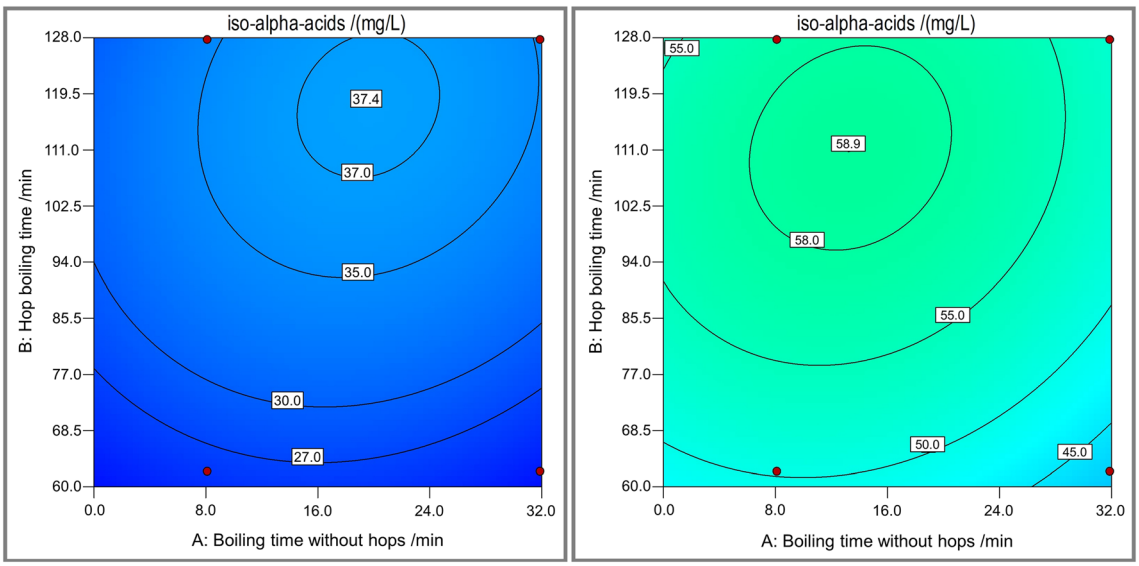

Figure 2. Iso-α-acids in boiled lauter tun wort at a dosage of 88 mg/L α-acids (left figure) and 172 mg/L α-acids (right figure). The dots represent the design boundaries. α=0.05, adjusted R^2=0.98. [Colour figure can be viewed at wileyonlinelibrary.com]

were used for the membrane mash filter trials as the number of plates limited the brew size. Mashing-in was carried out with a water to grist ratio of 3:1. The brewing program for the lauter tun and mash filter trials started at 62°C/20 min, 72°C/40 min and 78°C/2 min. The mashing-off for the continuous mash filtration system trials was performed at 72°C, due to the first wort extract with active α-amylase for post saccharification.

After mashing, the mash was separated with one of the lautering systems. The time required for the separation of wort and spent grains as well as the amount of sparging water was as follows: lauter tun: 120 min for 620 L, mash filter: 60 min for 250 L and continuous mash filtration system: 25 min for 620 L. Each system had a first wort concentration of almost 21.0°P. In the lauter tun and mash filter trials (Figure 1), the separated wort was transferred to the wort kettle and hop pellets (73 mg/L α-acids) were dosed at the start of boiling. The hops, Hallertauer Perle type 90 (2016), hop pellets with 7.8% α-acids were from Joh. Barth & Sohn GmbH (Nuremberg, Germany), and were boiled for 60 min.

The wort from the continuous mash filtration system was fractionated into three parts: 1% of the total wort as malt extract, 69% as boiling wort without hops and 30% as boiling wort with hops. The malt extract was separated from module 1 and stored in a small insulated vessel (Figure 1, '3a'). The wort boiled without hops represents the remaining wort of module 1 as well as the wort flow of module 2 and 3 and was boiled with an internal boiler (Figure 1, '3'). The hopped boiling wort, a low concentrate wort, represents the wort of module 4 and is boiled with an external boiler in a separate isomerisation vessel (Figure 1, '3b'). The boiling time and dosing quantity of α-acids correspond to the other trials of lauter tun and mash filter worts. After 60 min, the hopped wort was added to the unhopped wort, corresponding to the cast wort.

In the pilot brewery trials with the lauter tun, mash filter and continuous mash filtration system, the samples of the cast wort were cooled and transported (5°C) to the laboratory for analysis of bitterness (EBC), iso-α-acids (mg/L), α-acids (mg/L) and β-acids (mg/L).

Wort analyses

The preboiled worts at a pilot brewery scale were analysed for: pH with 206-pH1 from Testo SE & Co. KGaA (Lenzkirch, Germany); original gravity with DMA 35N from Anton Paar Germany GmbH, (Ostfildern-Scharnhausen Germany); fatty acids (C5-C10) (MEBAK - Wort, Beer, Beer-based Beverages (WBBM) 2.21.4 *(13)*); total soluble nitrogen (WBBM 2.6.1.1 *(13)*); calcium and magnesium (WBBM 2.24.12 *(13)*).

The cooled cast worts at a laboratory scale and 10 hL scale were analysed for bitterness (BU) (WBBM 2.17.1 *(13)*) and specific α-acids (mg/L), β-acids (mg/L) and iso-α-acids (mg/L) by HPLC. The wort samples were diluted 1:2 with distilled water and then centrifuged at 3000 g for 15 min at 20°C. Chromatography was performed with an UltiMate™ 3000 HPLC from Thermo Fisher Scientific GmbH (Dreieich, Germany) at a temperature of 35°C and a flow rate of 1.0 μL/min. An injection volume of 30 μL was used for iso-α-acids analysis and 20 μL for analysis of α- and β- acids. Two mobile phases were utilised (B and C) with B composed of 75% methanol, 24% 1mM EDTA and 1% o-phosphoric acid. For the mobile phase C only, methanol was applied. The elution started with 100% of the mobile phase B for the first 20 min. Mobile phase B was reduced to 80% and mobile phase C was increased to 20% for 8 min. The last 10 minutes of the analysis corresponded to 100% mobile Phase B. A Nucleodur® 100-5 C18 ec from Machery-Nagel GmbH & Co. KG (Düren) was selected for separation. Iso-α-acids were measured at 270 nm and α- and β-acids at 314 nm.

Results and discussion

There were significant variations between the isomerisation rates of the different worts from the lautering systems. Since the R^2 of the models are in agreement with the adjusted R^2 *(12)* (Table 2), the models of iso-α-acids, β-acids and bittering units are applicable. In addition, the respective F-values and p-values of <0.0001 show that the models are significant. The model of α-acids demonstrates a deviation of 0.06 between R^2 and the adjusted R^2.

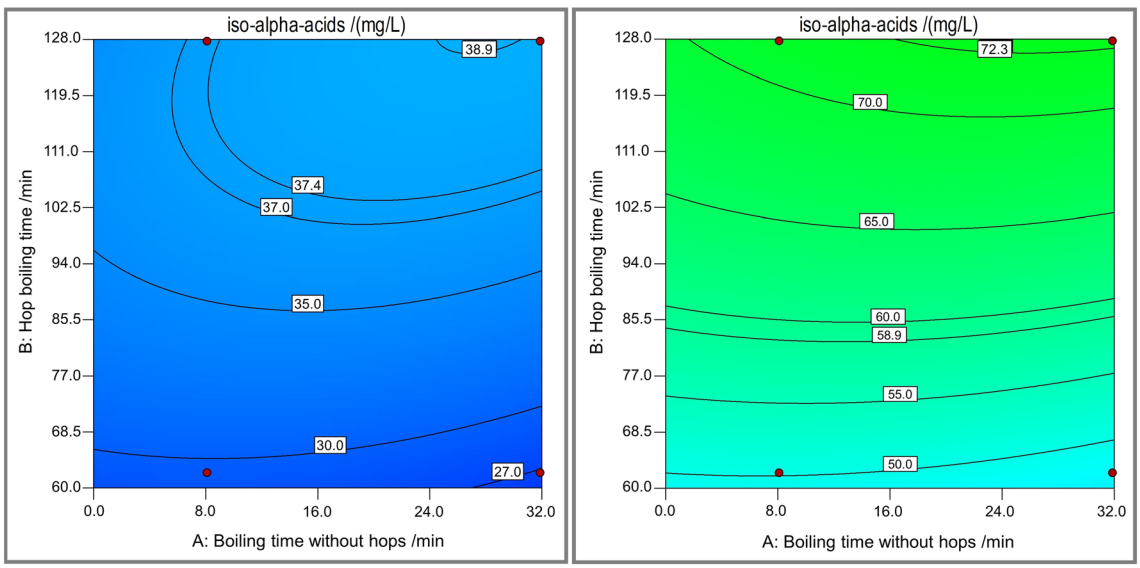

Figure 3. Iso-α-acids in boiled mash filter wort at an α-acid dosage of 88 mg/L (left figure) and 172 mg/L α-acids (right figure). The dots represent the design boundaries. α=0.05, adjusted R^2=0.98. [Colour figure can be viewed at wileyonlinelibrary.com]

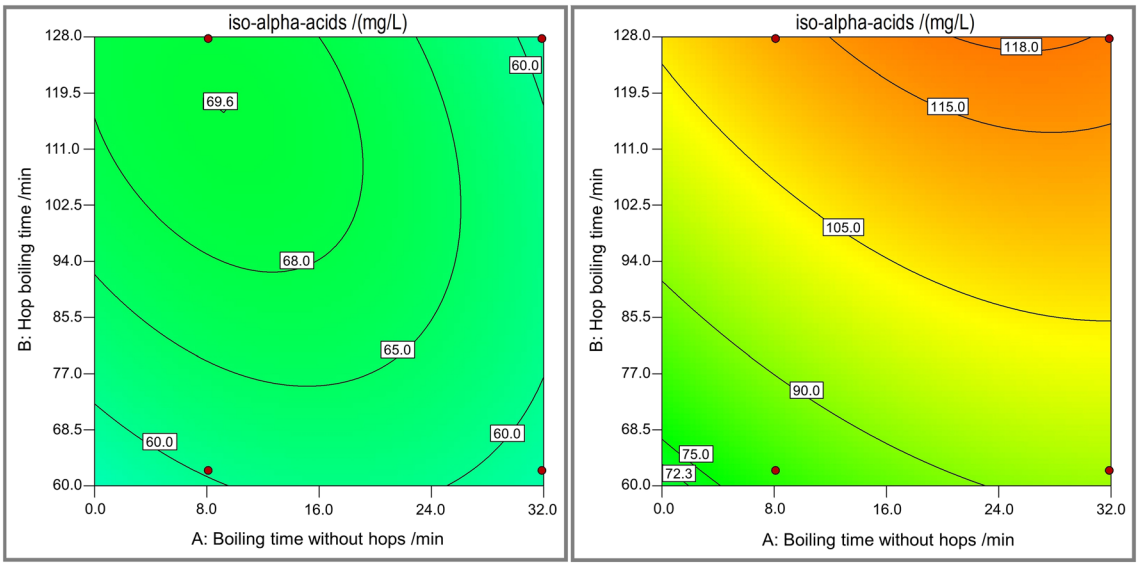

Figure 4. Iso-α-acids in boiled continuous mash filtration wort at a dosage of 88 mg/L α-acids (left figure) and 172 mg/L α-acids (right figure). The dots represent the design boundaries. α=0.05, adjusted R^2=0.98. [Colour figure can be viewed at wileyonlinelibrary.com]

However, the p-value of <0.0001 indicates that the model is significant. The probability that the α-acids model with the lowest model F-value of 16.30 is not significant is under 5%. In addition, all models had an insignificant lack of fit. This means that the variation of the model points does not differ significantly from the variation of the replicated points *(12)*. The model fitting applied can thus be utilised for the subsequent evaluation of the data.

The iso-α-acid contents of the worts (Figures 2–4) show that different boiling times are required for a specified hop isomerisation. Accordingly, the total boiling time equals the boiling time without hops (BTW) combined with the hop boiling time (HBT). To achieve an iso-α-acids content of 37.4 mg/L in the boiled wort at an α-acid dosage of 88 mg/L at start of boiling, the statistical evaluation for the lauter tun (LT) indicates a 20 min boiling time without hops (BTW) and 118 min boiling time with hops (HBT). The mash filter wort, requires 11 min BTW and 111 min HBT and for the wort from the continuous mash filtration system, 0 min BTW and 23 min HBT are required. This indicates a reduction of 12% in the total boiling time from lauter tun to mash filter and a reduction of 83% from lauter tun to continuous mash filtration system. Another example can be seen with an α-acid dosage of 172 mg/L and 58.9 mg/L iso-α-acids in the wort. Here, the boiling time reduction from lauter tun (total boiling time 124 min) to mash filter wort (total boiling time 84 min) is 32% and from lauter tun to continuous mash filtration system (total boiling time 44 min) about 65%. Consequently, higher α-acid dosages show a shift in the variation in boiling time between the lautering systems. The significant reduction in the required boiling time for worts from the continuous mash filtration system - in both examples - can be explained by the wort composition. Accordingly *(5,14)*, the rate of isomerisation increases in weak worts with a higher pH. In the experiments reported here, the wort from the continuous mash filtration system had, on average, an original gravity of 1.7 °P and a pH of 6.70, resulting in the reduction of the boiling time.

Malowicki and Shellhammer *(14)* reported that the change in pH does not directly affect the isomerisation rate, but that the solubility of α-acids is enhanced by an increase of pH. Accordingly, a lower original gravity correlates well with a decrease in protein content and trub formation, which is further illustrated by the values of total nitrogen in Table 3.

Table 4 reports the bitter substance composition of the boiled wort from different lautering systems. In addition to the increase in bittering units and the content of iso-α-acids from lauter tun (25.5 mg/L), mash filter (29.4 mg/L) to the continuous mash filtration system (60.3 mg/L), the amount of the residual α- and β-acids in the boiled continuous mash filtration system worts are noteworthy. Here, the α-acids in worts from the continuous mash system are 61% lower compared to the other lautering systems. This corresponds to the increased isomerisation rate. On the contrary, the content of the remaining β-acids in the worts from the

Table 3. Wort composition of the preboiled wort (95°C) from lauter tun (LT), mash filter (MF) and continuous mash filtration system (NE), n=3. With *s*: standard deviation, t_{tab}: value from t-table (4.30, for n=3 at a confidence interval of 0.05 *(15)*) and *n*: sample size.

		LT		MF		NE	
wort composition	unit	average	$\frac{s*t_{Tab}}{\sqrt{n}}$	average	$\frac{s*t_{Tab}}{\sqrt{n}}$	average	$\frac{s*t_{Tab}}{\sqrt{n}}$
original gravity	°P	12.5	0.61	12.8	0.41	1.7	0.2
pH	-	5.83	0.04	5.85	0.06	6.7	0.59
fatty acids (C5-C10)	mg/L	0.51	0.19	<0.04	-	<0.04	-
total soluble nitrogen	mg/100mL	102.8	9.63	108.4	11.66	16.1	4.26
calcium	mg/L	18.6	4.36	29.7	2.03	18.9	1.42
magnesium	mg/L	81.2	9.83	81.7	10.44	17.7	3.85

Table 4. Distribution of bitter substances at 8 min BTW, 62 min HBT, 88 mg/L α-acid dosage for lauter tun (LT), mash filter (MF) and continuous mash filtration system (NE). Adjusted R^2_{BU}=0.98, adjusted $R^2_{iso\text{-}\alpha\text{-}acids}$=0.98, adjusted $R^2_{\alpha\text{-}acids}$=0.88, adjusted $R^2_{\beta\text{-}acids}$=0.96.

boiling time without hops (min)	hop boiling time (min)	α-acid dosage (mg/L)	system	bittering units (EBC)	iso-α-acids (mg/L)	α-acids (mg/L)	β-acids (mg/L)
8	62	88	LT	40.5	25.5	21.1	0.6
			MF	43.6	29.4	27.1	0.7
			NE	69.3	60.3	10.6	27.6

continuous mash filtration system is about 40 times higher than the lauter tun and mash filter worts. Additional trials at a pilot brewery scale showed that post fermentation, the content of the β-acids in beers from continuous mash filtration system is comparable to those from lauter tun and mash filter despite the higher initial level in the wort. Consequently, the positive effect of β-acids on the bitter taste and the microbiological stability of beer *(5)* had no impact here.

The differences in the hop isomerisation rate in worts from the lauter tun and mash filter are remarkable as they have a similar pH and original gravity. For example, even with 8% higher bittering units and a 15% higher iso-α-acids content, the amount of α-acids is 15% higher (Table 4) in the mash filter wort, compared with that from the lauter tun.

Table 3 shows the values for original gravity, pH, fatty acids (C5-C10), total soluble nitrogen, calcium and magnesium in the unboiled, unhopped wort at 95°C. This illustrates the wort characteristics for hop isomerisation in the 5 L and 10 hL trials. The average original gravity of the lauter tun and mash filter worts is 12.7°P and the average pH is 5.84. As a consequence, the solubility of α-acids in the wort is reduced and thus, the isomerisation rate is lower in comparison to worts from the continuous mash filtration system *(16)*. The values for total soluble nitrogen and magnesium are similar for the two worts. However, the sum of C5-C10 fatty acids were 12 times higher in the lauter tun wort than in the mash filter wort. Further, the level of calcium was 59% higher in mash filter compared to lauter tun wort. According to the patent of Atlantic Research Institute *(17)*, calcium has a catalytic effect on hop isomerisation. This would explain the higher yield of bitterness in the mash filter wort. However, Malowicki and Shellhammer *(14)*, showed that calcium (100 mg/L calcium chloride) had no significant effect on the concentration of iso-α-acids in an aqueous solution at pH 5.2. Accordingly, it is questionable whether the calcium concentration of 18.6 mg/L in lauter tun wort and 29.7 mg/L in mash filter wort had any effect on isomerisation. It is known that the hop bitter substances bind to proteins via the ε-amino groups of lysine and are involved in the formation of trub *(18)*. In addition, long chain fatty acids associate with the trub due to their hydrophobicity *(19)*. The question arises, whether the short/medium chain fatty acids in wort lead to an increase in trub formation or only bind to pre-formed trub, as is the case with long-chain fatty acids. Consequently, the short/medium chain fatty acids may be responsible for losses of hop bitter substances in the hot wort.

The experiments at a 5 litre scale show that different wort composition have an influence on the isomerisation rate. In addition, it is possible to increase the yield of iso-α-acids through optimisation of the boiling time. Of course, other considerations in wort boiling such as the evaporation of dimethyl sulphide and achieving the desired original gravity should be considered further.

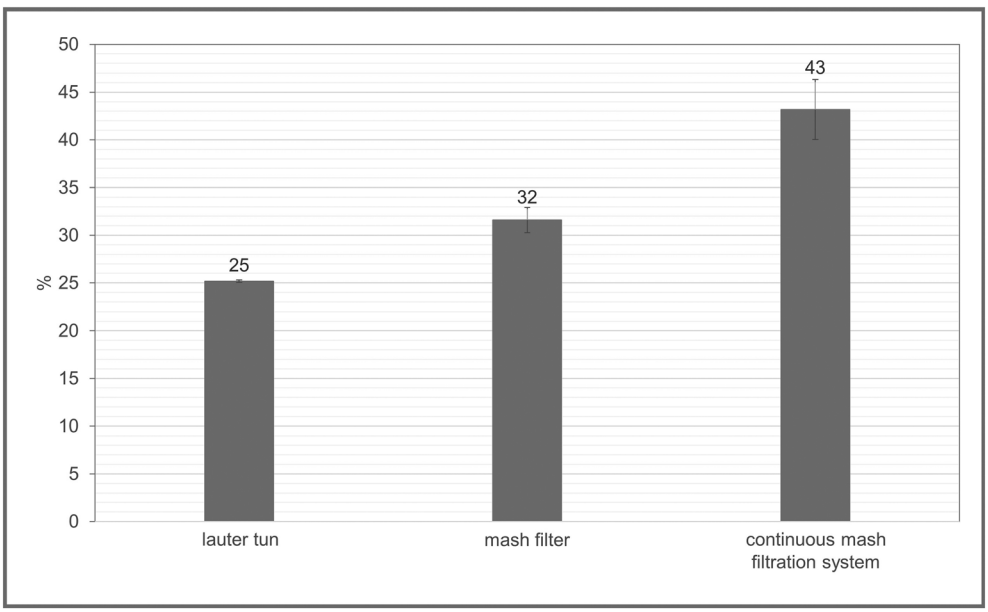

Figure 5. Utilisation of iso-α-acids up to cast wort in a pilot brewery. The error indicators represent the standard deviations of the calculated utilisation values. LT= lauter tun, MF=mash filter, NE=continuous mash filtration system. n=2.

In order to confirm the results of the laboratory scale experiments, six brews were performed at a larger scale in a pilot brewery. Figure 5 illustrates the utilisation (Eq 1) of iso-α-acids of the three different worts up to casting the wort. Due to the wort composition, the continuous mash filtration system wort shows the highest utilisation with 43% after 60 min boiling time and the same α-acid dosage, compared to 25% for the lauter tun and 32% for the mash filter. In the continuous mash filtration system, the concentration of α-acids is higher in the vessel (Figure 1, '3b'), compared to that in the trials with the lauter tun and mash filter. Calculations of hop isomerisation show the α-acid dosage was as follows: 73 mg/L for the lauter tun and mash filter wort with 246 mg/L for the continuous mash filtration system wort in the separate vessel. Although the higher concentration negatively influences the isomerisation rate *(20)*, a higher yield is achieved. The results show that at around pH 7.0 and an original gravity of ca. 1.7 °P, higher α-acid (>200 mg/L) dosage does not result in major losses, because of the lower amount of interfering substances and the better solubility of α-acids.

Conclusion

The work reported here shows that the same hop yields for continuous mash filtration system, mash filter and lauter tun can only be achieved by extending the boiling time. The total boiling time according to the lautering systems increases as follows: continuous mash filtration system < mash filter < lauter tun. Further, the different boiling times without hops must be considered prior to the hop boiling time.

Accordingly, it is suggested that it is possible to determine the optimal parameters (hop quantity, hop dosage times, boiling time) for each brewery with regard to hop isomerisation. Successful optimisation of this process would enable savings in hops through higher isomerisation rates. With the development of a modelling tool, the parameters can be determined individually, and would be valid for all sizes and design of breweries.

Acknowledgements

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Conflicts of interest

The authors declare there are no conflicts of interest.

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